Standard Operating Procedure for the Determination of Total Phosphorus and Total Kjeldahl Nitrogen

1.0 Scope and Applicability

This method is applicable to drinking and surface water, domestic and industrial waste. The applicable range is 0.02 to 5.0 mg P/L and 0.1 to 5.0 mg N/L. Both curves are linear. The MDLs for phosphorus and TKN are 0.016 mg P/L and 0.024 mg N/L. The MDL were developed using spikes into reagent grade (17 meg-ohm) water. Total phosphorus may also be measured by using the ammonium persulfate digest method (SOP I-1-12). Results for phosphorus from the TKN digest may yield slightly higher values due to the longer and more rigorous digestion.

2.0 Summary of Method:

2.1 Phosphorus occurs in several forms: ortho, poly and organically bound. Further classification can be made on whether it is dissolved. This last classification is artificial and defined as passing through a 0.45 micron filter. The various forms and classifications are outlined in section 18.0. In the TKN digest, sulfuric acid with a copper sulfate catalyst is boiled to convert the various phosphorus forms to the ortho form and will produce a result known as total phosphorus or total dissolved phosphorus if the sample has been filtered. Ortho phosphorus must be determined with direct color development without a digestion step and within 48 hours, see SOP I-1-12). After the sulfuric acid digest the ortho phosphorus will react with ammonium molybdate and antimony potassium tartrate to form an antimony-phospho-molybdate complex. This is reduced to a blue-colored complex by ascorbic acid, which will absorb light at 880 nm. Alternatively Total Phosphorus may be measured alone using a persulfate digest (see persulfate method (SOP I-1-12).

.

2.2 Nitrogen like phosphorus may exist in several forms: as the ammonium ion (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), organic bound, azo (-N=N-), amine (-NH_2) etc. The block digester uses sulfuric acid with a copper sulfate catalyst and K_2SO_4 to raise the boiling point to break down the various forms to ammonium sulfate. The term Total Kjeldahl Nitrogen is somewhat of a misnomer as the digestion as it is used here is not a true Kjeldahl and does not give total Nitrogen. Simple nitrogen compounds like ammonium compounds and organic forms will form ammonium salts. Nitrates and other forms may not break down to ammonium sulfate.

Approximately 0.2 ml of this digested sample is injected onto the chemistry manifold where its pH is controlled by raising it to a known, basic pH with a concentrated buffer. This in-line neutralization converts the ammonium cation to ammonia, and

also prevents undue influence of the sulfuric acid matrix on the pH sensitive color reaction which follows.

In Lachat's copper catalyst Method "H"(June 95), the ammonia thus produced is heated with salicylate and hypochlorite to produce a blue color which is proportional to the ammonia concentration. The color is intensified by adding sodium nitroprusside. The presence of EDTA in the buffer prevents precipitation of calcium and magnesium. The intensity of the color can be measured at 660 nm. An alternative method to measuring the ammonium ion is to use alkaline phenol and intensify the color with nitroprusside without the salicylate. This is the procedure we will use here. Lachat's Method 10-107-06-2-H uses a carrier consisting of the digestion reagent which contains sulfuric acid then Lachat neutralizes it at the next step by using 0.8 M sodium Hydroxide. This is waste of chemicals and doesn't serve any purpose. The acid carrier will be replaced with distilled water. The little bit of color attributed to the copper sulfate in the digestion mixture, which will now be missing when distilled water is used in its place, has been found to offer little matrix effect. This approach is used with Lachat's method 10-107-06-1-B (Dec 1993) for the analysis of ammonia. The manifold diagram and recommended pump tubes for method "B" will be used. The more concentrated buffer of the H method will be retained in order to handle the acid left after the block digestion step. Some problems with staining of lines, and an elevated baseline after the chemistry has been running for a while have been found to be associated with the concentration of the nitroprusside reagent. This reagent has been reduced in strength to 0.5 mg/L.

3.0 Definitions:

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

TKN total kjeldahl nitrogen
FIA flow injection analysis
LMS Laboratory Management System
MSDS Material Safety Data Sheet

4.0 Interferences:

Samples for TKN digest must not consume more than 10% of the sulfuric acid during the digestion. The buffer is designed to accommodate slight changes in acid content.
Silica forms a pale blue complex which also absorbs at the phosphate peak of 880 nm. This interference is generally insignificant as a silica concentration of approximately 4000 ppm would be required to produce a 1 ppm positive error in orthophosphate.

5.0 Safety

WARNING: The major concern with this method is the use of phenol. The following health hazard data has been listed for phenol in the MSDS. Absorbed rapidly through skin, burns skin severely, may be fatal if absorbed through skin, irritates eyes, burns eyes, dermatitis, irritates nose and throat, headache, dizziness, gastrointestinal disturbances, convulsions, liver damage kidney damage, blindness, Death has resulted in 30 minutes to several hours from absorption of phenol through a skin area of as little as 64 square inches. cyanosis, ochronosis. To minimize exposure to phenol were latex gloves when making reagents containing phenol and run water down the drain when the reagent is in use to minimize vapor in the air.

This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

6.0 Equipment and Supplies:

Note: Brand names, suppliers, and part numbers are cited for illustrative purposes only. No endorsement is implied. Equivalent performance may be achieved using equipment and materials other than those specified here, but demonstration of equivalent performance that meets the requirements of this method is the responsibility of the laboratory.

6.1 Lachat QuikChem 8000 Automatic Flow Injection Analyzer

6.1.1 XYZ autosampler

6.1.2 proportioning pump

6.1.3 Injection module

6.1.4 Colorimeter with:
10 mM flow cells
880 nm interference filter for PO4
660 nm interference filter for TKN

6.1.5 Phosphate and TKN reaction modules

6.1.6 sample loops: 25 cm for TKN and 25 cm for PO4

6.1.7 Heating coils: 60 deg C for TKN and 37 deg C for PO4

6.1.8 Gateway 2000 P5-60 computer with omnion Ver. 2.0 software running under windows.

6.1.9 NEC MultiSync 3FGe monitor

6.1.10 HP 8150 printer

6.2 Lachat 46 place block digestor

7.0 Reagents and Standards:

All reagents are ASC Reagent grade or higher.

7.1 PHOSPHATE REAGENTS

7.1.1 Stock Ammonium Molybdate Solution
In a 1L volumetric flask dissolve 40.0 g ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\bullet 4H_2O$] in approximately 800 ml of water. Dilute to the mark and mix with a magnetic stirrer for at least four hours. Store in plastic and refrigerate.

7.1.2 Stock Antimony Potassium Tartrate Solution
In a 1L volumetric flask, dissolve 3.0 g antimony potassium tartrate (potassium antimony tartrate hemihydrate $K(SbO)C_4H_4O_6\bullet 1/2H_2O$ in approximately 800 ml water. Dilute to mark and mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

7.1.3 Molybdate Color Reagent
In a 2L flask, add about 1L of water. Add 144 ml of stock antimony potassium tartrate solution and 426 ml of stock ammonium molybdate solution. Dilute to the mark and stir until mixed. Degas with Helium.

7.1.4 Ascorbic acid
In a 1L beaker dissolve 60.0 g ascorbic acid in about 900 ml of water. Degas with Helium. Add 1.0 g of sodium dodecyl sulfate ($CH_3(CH_2)_{11}OSO_3Na$). Mix with a stir bar and dilute to the 1 liter mark. Prepare fresh weekly more frequently if problems arise.

7.1.5 Diluent

In a 1L volumetric flask, dilute 240 ml of Digestion Solution to 1L with water.

7.1.6 Carrier
PO4: In a 2L flask dissolve 63.4 g K_2SO_4, and 55 ml conc. H_2SO_4
TKN: distilled water

7.1.7 Sodium Chloride/Sodium Hydroxide Soln.
In a 2L beaker dissolve 320 g NaCl and 40 g NaOH in about 1200 ml of water. Dilute to the 2 liter mark and degas with Helium.

7.2 TKN REAGENTS

7.2.1 TKN Buffer
Combine 100 g NaOH, 27.2 g EDTA, 48 g Sodium Phosphate dibasic heptahydrate ($Na_2HPO_4 \bullet 7H_20$) in about 1600 ml water. Stir to dissolve and dilute to 2L. This reagent appears to be near the saturation point and may precipitate in time.

7.2.2 Nitroprusside Color Reagent
Add 1.0 g of sodium nitrprusside (sodium nitroferricyanide diydrate ($Na_2Fe(CN)_5NO \bullet 2H_2O$)) in about 800 ml water. Stir to dissolve and dilute to 2L. Store in dark bottle and prepare fresh monthly. Degas with Helium.

7.2.3 Hypochlorite solution
Combine 500 ml of regular Clorox Bleach (5.25% sodium hypochlorite) with enough water to make 1L. Mix and degas.

7.2.4 Sodium Phenolate
Warning: Wear gloves, skin burns can occur and phenol can be rapidly absorbed through the skin. In a two liter beaker pour 176 ml of liquefied phenol into 1200 ml of distilled water. While stirring, slowly add 64 g of Sodium Hydroxide (NaOH). Cool and dilute to 2 L. Degas with Helium.

7.2.5 Digestion Solution
In a 3 L flask add 2000 ml water, add slowly 268 ml conc. sulfuric acid, 268 g potassium sulfate (K_2SO_4). Add 14.6 g Copper Sulfate ($CuSO_4 \cdot 5H_2O$) and dilute to the 3 L mark. Use 7.5 ml of this solution for each digestion tube. This solution has been diluted from the original recipe to prevent freeze up of the repipeter.

7.2.6 Carrier line will use distilled water.

7.2.7 Hydrochloric acid rinse solution
Make a 50/50 solution with conc. HCl and deionized water in a beaker. Rinse lines with this solution to removed staining due to the color reagents.

7.3 QUALITY CONTROL REAGENTS

7.3.1 Combined Stock Standard (1000 ppm N & 1000 ppm P)
In a 1L volumetric flask add 3.8188 g NH_4Cl and 4.3938 g KH_2PO_4. Mix and dilute to mark.

7.3.2 Working Standard (20 ppm N, 20 ppm P)
Dilute combined stock standard 4 ml to 200 ml. Use this standard to add to the 75 ml tubes in the block digester.

STD	ml working standard	ppm N	ppm P
5	5.0	5.0	5.0
2	2.0	2.0	2.0
1	1.0	1.0	1.0
.5	0.5	0.5	0.5
.1	0.1	0.1	.1
0	0	0	0

7.3.3 Stock Spike Solution (500 ppm N, 500 ppm P)
To a 1L volumetric flask, add 1.9094 g NH_4Cl and 2.1968 g KH_2PO_4. Mix and dilute to the mark.

7.3.4 Working Spike Solution (25 ppm N, 25 ppm P)
Dilute the stock spike solution 1:20. Use 0.40 ml of this to obtain a spike of .5 ppm N and .5 ppm P.

7.3.5 Check solution (100 ppm N from nicotinic acid)
Place 0.8614 g of nicotinic acid 98% pure in a 1 liter volumetric flask and add water to the mark. Use a 1 ppm solution as a check. Alternatively a Quality Control sample like a DMR or WS (water supply) with established know values and ranges could be used.

7.4 Other items

7.4.1 Chemware PTFE boiling stones; Norton Co. (part number A1069103)

8.0 Sample Collection, Preservation, and Storage

Samples for TKN and Total Phosphorus should be preserved with H_2SO_4 to a pH <2 and stored at 4 deg C. Acid preserved samples have a holding time of 28 days. Samples digested should be run on the FIA within one week of digestion.

9.0 Quality Control

Each sample run of 46 tubes will include 1 check (1 ppm nicotinic acid), 3 duplicates, 4 spikes and a control sample of known value and range. Standards will be treated as samples and digested along with the samples. The remaining tubes being devoted to samples, and possibly blank (water) digestions. Current data regarding two and three St. Dev. as Quality Control marks will be used and the set rerun if more than two quality control samples fail. TKN have a poorer precision than phosphate samples. It is because of this that 3 duplicates and 4 spikes are required in order to arrive at an evaluation on how well the samples ran. Spike recoveries of 60-140% appear to be normal (+/-2σ). It is hoped that improved values for precision can be obtained with further work. The main concerns on Data Quality for TKN's center around the block digestion step, although considerable variability has been seen due to the chemistry on the FIA manifold.

Spiked samples are prepared by placing 0.4 ml of the 25 ppm spike solution into the sample tubes which are spiked. When the final volume is adjusted to 20 ml after the digestion step the effective spike amount will be 0.5 ppm for both TKN (N) and PO_4 (P).

10.0 Calibration and Standardization:

Standards are prepared according to the outline in section 7.3.2. There are 5 standards and a zero standard. The standards will be used externally to the samples and will always run with the samples. Some baseline drift is common with this method and an internal check under software control will check the value of the high standard at intervals of 25 samples or less. If the high standard is found to be out of range by more than 8% the Standards will be rerun and the previous samples re-analyzed. If the high standard is within 5% error then the software will direct a re-calibration and a continuation of the analysis. For the standard calibration to pass the coefficient of determination will need to be 0.995 or better. Both curves are linear.

11.0 Procedures:

There are two steps involved with PO4 and TKN analysis. These are: preparation and digestion on the block digestor and the color development and analysis on the Lachat FIA.

11.1 Digestion Procedure

11.1.1 Both samples and standards should be carried through the block digestion.

11.1.2 Add 4 to 8 Teflon boiling stones (8.4.1) to each tube.

11.1.3 Add 7.5 ml of digestion solution to each of the 75 ml tubes.

11.1.4 Add 20 ml to each tube for samples, duplicates and spiked samples. If the ammonia value for the sample is available and is over the 5 ppm (top standard) use an aliquot which would place the result at mid range. For example on a sample running 19 ppm NH_3, use a 2.00 ml aliquot (a 1:10 dilution at this point). This is preferable to having to dilute on the manifold. All discharge samples should be run 1:10 through the block digestor as they are frequently over range.

11.1.5 Add the spikes and check samples to the set as laid out in the work list.

11.1.6 Place the tubes into a preheated block at 160 deg C. Place the cooling fingers on top and start the second stage of heating to 380 deg C for 2.5 hrs. The EPA procedure calls for a 1 hour cook off of the water at 160 deg followed by a ramp to 380 and holding there for 2 ½ hours. In actual practice after receiving the Lachat block with the modified holes, it was no longer possible to drive out the water at 160 deg. Ramping to 380 deg. Does not appear to be a problem as the water is lost on the way up.

11.1.7 After the digestion step is completed, remove the tubes and place them in the holder. Allow tubes to cool slightly.

11.1.8 When tubes can be safely handled by hand, (WARNING: tubes are very hot and will give off puffs of sulfuric acid), remove the coggles and add 19 ml of deionized water to each tube. Tubes should be very warm at this point but not so hot as to burn your hand. Vortex all tubes to dissolve any salts. If the tubes sit to long they may need extensive vortexing, warming or ultrasonic treatment to bring crystalline materials into solution.

11.1.9 Samples are ready for the FIA when all materials are in solution. Tubes may also be held for 1 week if capped or covered tightly.

11.2 FIA Procedure

11.2.1 Fifteen minutes prior to analysis, turn on the computer which will turn on the heaters. Connect the necessary interference filters, reaction modules and sample loops. Make sure the heaters are in line.

11.2.2 Assemble all the pump tubes for each manifold according to the diagrams.

11.2.3 Pump water through the lines and check for leaks.

11.2.4 Place all standards and samples into appropriate trays.

11.2.5 Degas all reagents taking care to use the specially designated PO4 degassing line only for the NaCl phosphate carrier line. CAUTION: The TKN buffer contains PO4 and will contaminate the degassing tubes if not careful.

11.2.6 Place all feed lines into their respective reagent bottles according to the TKN and PO4 manifold setup drawings. (see SECTION 18.0)

11.2.7 Load the proper method, Tray and DQM plan. Start the run and observe the baselines on the monitor. If the baselines are flat: continue: if not restart the run and observe again.

11.2.8 At the end of the run, rinse all feed lines for 5 min. in deionized water.

11.2.9 Rinse both TKN and PO4 lines in the HCl solution prepared in 8.2.7 for 5 min. Rinse with distilled water for 5 min, pump dry and remove the pressure from the pump tubes.

12.0 Data Analysis, Calculations, and reporting results

All calculations will be made by the FIA computer using a least squares regression based upon digested standards ran at the same time as samples. Results will be reported in mg P/L and mg N/L.

13.0 Method Performance:

The method detection levels were calculated using 8 replications of 0.05 ppm N and P. The MDL for P was 0.016 with a precision of 0.00506 and the MDL for N was 0.024 with a precision of 0.0083 mg/L. This compares with a performance detection level (PDL) of 0.1183 ppm for TKN using 23 duplicates with a range of 0.1 to 0.5 ppm and a PDL of 0.0162 ppm for P using 41 duplicates with a range of 0.018 to 0.1 ppm P. This calculation procedure using duplicate data for a measure of precision is explained in Standard Methods for the Examination of Water and Wastewater, 18th Edition, 1992.

14.0 Pollution Prevention:

The present TKN method does away with the mercury catalyst used in past methods. This is somewhat of an improvement although its replacement, copper, also has some environmental problems.

15.0 Waste Management:

The use of phenol also contributes to air pollution within the lab as well as a disposal concern.
The TKN chemistry is frequently plagues with contamination of reagents (phenol being one) resulting is premature disposal of chemicals before they are fully consumed. Preparing amounts consistent with current sample loads helps with waste disposal.

For further information on waste management consult The Waste Management Manual for Laboratory Personnel and Less is Better: Laboratory Chemical Management for Waste Reduction, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036

16.0 References

16.1 Phosphate

16.1.1 Lachat QuikChem Method 10-115-01-1-C "Total Phosphorus in Kjeldahl Digests" Oct 1994.

16.1.2 EPA (Mar 83) Method 365.4 (Colorimetric, Automated, Block Digester AAII).

16.2 Total Kjeldahl Nitrogen

16.2.1 Lachat QuikChem Method 10-107-06-2-H "Total Kjeldahl Nitrogen with Copper by FIA" June 1995.

16.2.2 EPA (Mar 83) Method 351.2 (Colorimetric, semi-Automated Block Digester, AAII).

16.2.3 Lachat QuikChem Method 10-107-06-1-B "Determination of Ammonia (Phenolate) by Flow Injection Analysis Colorimetry" Dec 1993.

17.0 Tables, Diagrams, Flowcharts, Validation Data and Additional Information

17.1 Raw data to be saved include the print out of the mg N/L and mg P/L of the samples along with the printing of the regression analysis of the standards. After the data has been entered into the LIM System, the distribution sheet will also be kept. All records will be kept in the PO4/TKN book in order of the date of analysis.

Types of Phosphorus

Analyte#	analyte	.45 µm filtered	digest
9408	dissolved ortho	yes	No

9410	total ortho PO4	No	No
9412	total dissolved PO4	Yes	Yes
9415	total PO4	No	Yes

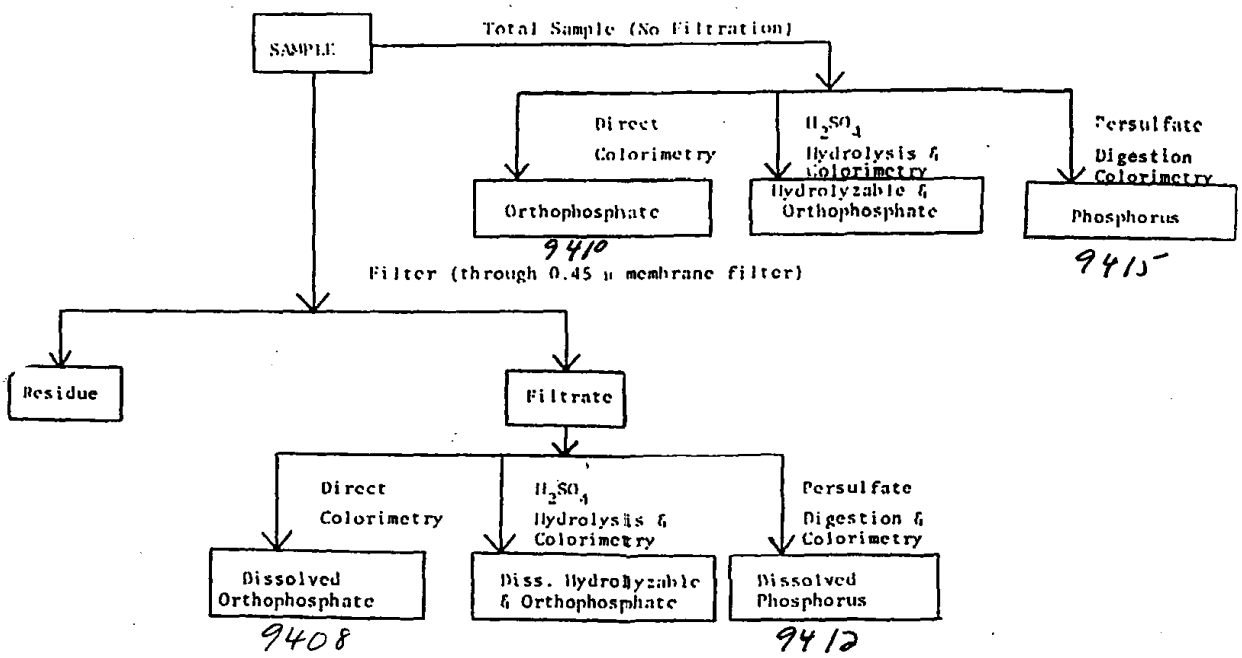

Figure 1. Analytical scheme for differentiation of phosphorus forms

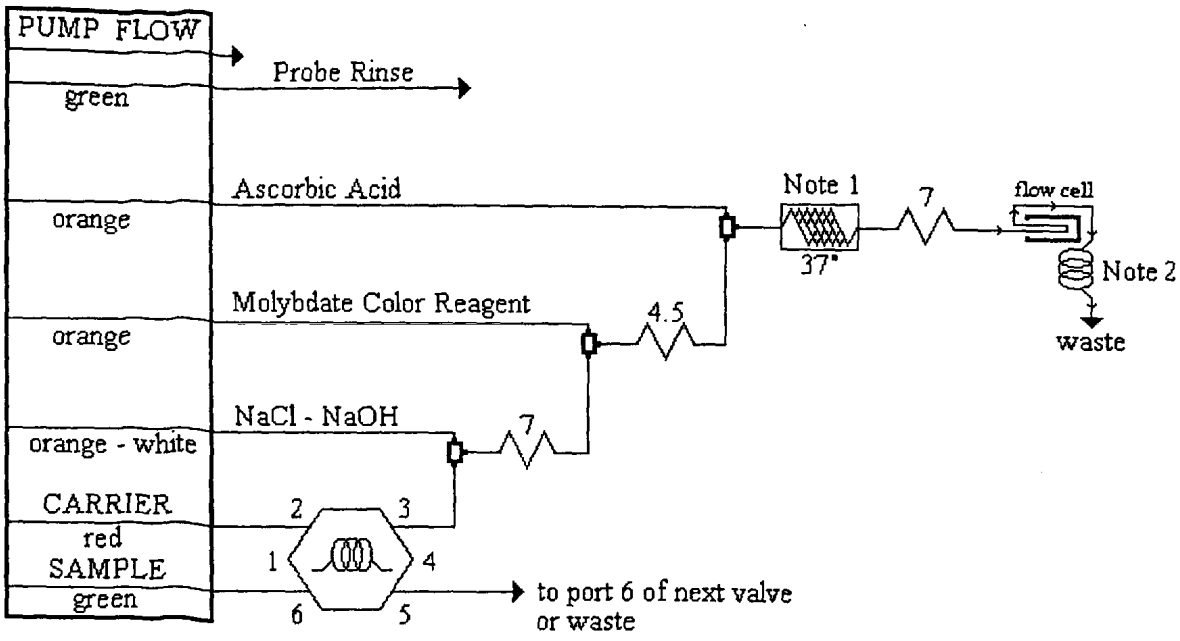

Figure 2. Phosphate Manifold diagram

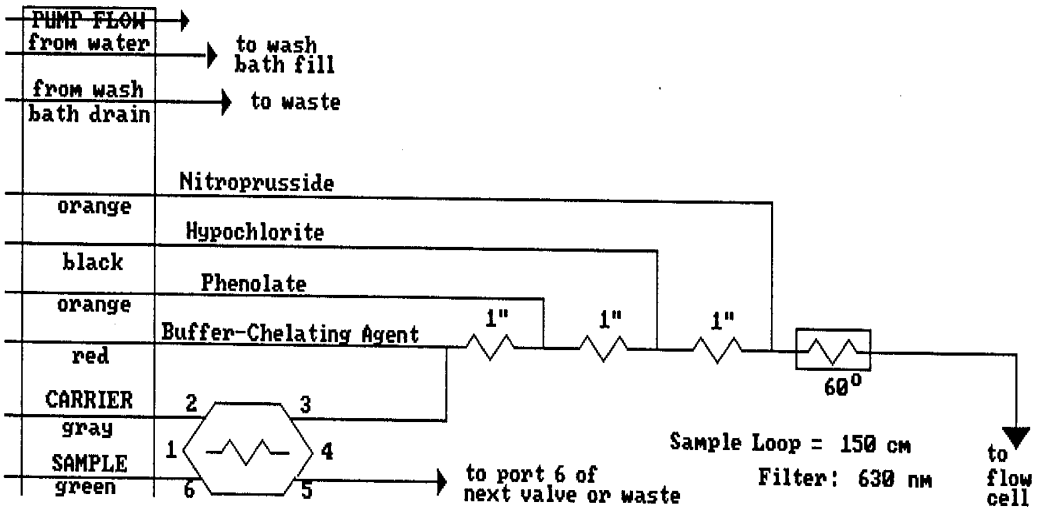

Figure 3. TKN Manifold diagram